

### **2.3. Mobile phase and diluent**

The mobile phase was prepared by combining volumes (as indicated below) of distilled water with methanol, acetonitrile (1.58:1:1) and triethylamine(2 drops) . The pH was adjusted using phosphoric acid. The mobile phase was filtered through a 0.45 µm filter membrane and degassed by sonication. The diluent was prepared by adding distilled water, acetonitrile and methanol in equal volumes (1:1:1).

### **2.4. Stock solutions**

All stock solutions of amlodipine, atorvastatin, glimepiride were prepared by dissolving 25 mg of each drug in 80 ml of diluent (in 100 ml volumetric flask) with sonication for 20 min. The volume was adjusted to 100 ml with the same diluent. Then 10 ml was taken and diluted up to 50ml. (total conc. =0.05)

### **2.5. Extraction of drugs from human plasma**

Human plasma obtained from Al-Salt hospital. Plasma (10 ml) was mixed with 4 mg of glimepiride, 5 mg of amlodipine and 20 mg of atorvastatin, incubated for 30 min and then extracted. The extraction process was performed by adding 20 ml of equal mixture of ether and ethyl acetate mixture. This is followed by adding 2 drops of NaOH and then transferred to a separating funnel, incubated for 15 min until full separation occurred. This process was repeated for four times on the same plasma. The resulted extraction was filtered and then evaporated at low temperature (30°C). Following the evaporation, 50 ml of diluent were added, filtered and measured. (conc. =0.08, 0.1, 0.4 respectively)

### **2.6. Method precision solution preparation**